PATENT

Docket No. 2629-4005US1

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

UTILITY APPLICATION AND APPLICATION FEE TRANSMITTAL (1.53(b))

ASSISTANT COMMISSIONER FOR PATENTS
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Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

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For: UNIVERSAL COLLECTION MEDIUM

Enclosed are:

[X] 23 page(s) of specification, 1 page(s) of Abstract, 4 page(s) of claims

[ ] 11 sheets of drawing [ ] formal [x] informal

[ ] 6 page(s) of Declaration and Power of Attorney

[X] Unsigned
[ ] Newly Executed
[ ] Copy from prior application

[ ] Deletion of inventors including Signed Statement under 37 C.F.R. § 1.63(d)(2)

[ ] Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the combined declaration and power of attorney is supplied herein, is considered as being part of the disclosure of the accompanying application and is incorporated herein by reference.

[ ] Microfiche Computer Program (Appendix)

[ ] _____ page(s) of Sequence Listing

[ ] computer readable disk containing Sequence Listing
[ ] Statement under 37 C.F.R. § 1.821(f) that computer and paper copies of the Sequence Listing are the same

[X] Claim for Priority

[ ] Certified copy of Priority Document(s)

[ ] English translation documents

[ ] Information Disclosure Statement

[ ] Copy of ____ cited references

[ ] Copy of PTO-1449 filed in parent application serial No._____.

[ ] Preliminary Amendment

[X] Return receipt postcard (MPEP 503)

[ ] Assignment Papers (assignment cover sheet and assignment documents)

[ ] A check in the amount of $40.00 for recording the Assignment.

[ ] Assignment papers filed in parent application Serial No. _____.

[ ] Certification of chain of title pursuant to 37 C.F.R. § 3.73(b).

[ ] This is a [ ] continuation [ ] divisional [ ] continuation-in-part (C-I-P) of prior application serial no. _____.

[ ] Cancel in this application original claims _____ of the parent application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

[ ] A preliminary Amendment is enclosed. (Claims added by this Amendment have been properly numbered consecutively beginning with the number following the highest numbered original claim in the prior application.

[ ] The status of the parent application is as follows:

[ ] A Petition For Extension of Time and a Fee therefor has been or is being filed in the parent application to extend the term for action in the parent application until _____.

[ ] A copy of the Petition for Extension of Time in the co-pending parent application is attached.

[ ] No Petition For Extension of Time and Fee therefor are necessary in the co-pending parent application.

[ ] Please abandon the parent application at a time while the parent application is pending or at a time when the petition for extension of time in that application is granted and while this application is pending has been granted a filing date, so as to make this application co-pending.

[ ] Transfer the drawing(s) from the patent application to this application.

[ ] Amend the specification by inserting before the first line the sentence:
This is a [ ] continuation [ ] divisional [ ] continuation-in-part of co-pending application Serial No._____ filed _____.

I. CALCULATION OF APPLICATION FEE (For Other Than A Small Entity)

					Basic Fee
	Number Filed		Number Extra	Rate	$760.00
Total Claims	27	-20=	7	x$18.00	$126.00
Independent Claims	4	- 3=	1	x$78.00	$ 78.00
Multiple Dependent Claims	[ ] yes [X] no		Additional Fee = Add'l Fee =	$260.00 NONE	$0

Total: $ 964.00

[ ] A statement claiming small entity status is attached or has been filed in the above-identified parent application and its benefit under 37 C.F.R. § 1.28(a) is hereby claimed. Reduced fees under 37 C.F.R. § 1.9(F) (50% of total) paid herewith $ _____.

[ ] A check in the amount of $ _____ in payment of the application filing fees is attached.

[X] Charge Fee(s) to Deposit Account No. 13-4500. Order No. 2629-4005US1. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

[X] The Assistant Commissioner is hereby authorized to charge any additional fees which may be required for filing this application, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2629-4005US1. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

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Dated: December 11, 1998

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Docket No.2629-4005US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Attila T. Lorincz, et. al.	Group Art Unit:	TBA
Serial No.	:	TBA	Examiner:	TBA
Filed	:	11 December 1998		
For	:	**UNIVERSAL COLLECTION MEDIUM**		

EXPRESS MAIL CERTIFICATE

Express Mail Label No. EJ557774681US

Date of Deposit 11 December 1998

I hereby certify that the following attached paper(s) and/or fee

Utility Application and Application Fee Transmittal 91.53(b)), duly executed; Unexecuted Combined Declaration And Power of Attorney; 23 pages of the Specification, 1 page of the Abstract, 4 pages of Claims (1-27), and 11 Sheets of informal Drawings; Return Receipt Postcard, and an Express Mail Label bearing Express Mail No. EJ557774681US.

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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FORM: EXP-MAIL.NY
Rev. 05/27/98

2629-4005US1

United States Patent Application

for

Universal Collection Medium

UNIVERSAL COLLECTION MEDIUM

FIELD OF THE INVENTION

The present invention is generally related to the field of cytological and molecular assays and specifically to the area of assays for the assessment of conditions using cytological and molecular assays.

BACKGROUND OF THE INVENTION

The detection and diagnosis of human conditions is of obvious importance for the treatment of disease. Numerous characteristics of diseases have been identified and many are used for their diagnosis. Many diseases are preceded by, and are characterized by, changes in the state of the affected cells. Changes can include the expression of viral genes in infected cells, changes in the expression patterns of genes in affected cells, and changes in cell morphology. The detection, diagnosis, and monitoring of diseases can be aided by the assessment of such cell states.

Routinely, for patients suspected of having one or more infectious diseases, for example human papilloma virus or herpes simplex virus, a sample of cells is taken from the patient for analysis. Generally, such a sample is in the form of a swipe or cellular scrape from the area primarily affected by the disease. These swipes usually collect a mixture of normal and diseased cells with a very limited total number of cells. The collected cells are traditionally smeared onto a slide for further analysis. When biochemical analysis was attempted, it was done at the expense of a cytological analysis and was done via qualitative methods such as *in situ* hybridization.

Routinely, the cervical sample obtained for conventional cytology is smeared onto a slide for morphological analysis. If this sample identifies potential disease by cell cytology, the patient must return for colposcopy to have a second sample collected for repeat cytology and/or genetic analysis and other molecular tests such DNA, RNA or protein. Recently, liquid cytology media have appeared on the market, which provide for enhanced morphology. These media were discovered to be

amendable to molecular tests such as for HPV DNA, however, cells are routinely collected into 10-20 ml of preserving agents, which excessively dilute DNA, RNA and other asssayable biomolecules, making molecular testing less than ideal. Further, while the current preserving reagents preserve cellular morphology these reagents allow degradation of DNA and RNA, such that quantitative analysis becomes difficult or impossible upon storage.

Thus, present day analysis requires at least two samples to be obtained from a patient in order to determine cell morphology and quantitative genetic analysis. Current cytology methods use large volumes of a preserving agent which excessively dilute DNA, RNA and other assayable biomolecules. Further, while the current preserving reagents preserve cellular morphology these reagents allow degradation of individual biomolecules such as DNA, RNA and protein, such that quantitative analysis becomes impossible.

Designing methods and media to preserve cells or tissue for analysis by both morphological and biochemical analysis has proven problematic in the past. For example, fixing cells or tissue for morphological analysis compromised the ability to do biochemical analysis. Preserving cellular RNA was done at the expense of DNA and morphological analysis. Similarly, preserving cellular DNA was done at the expense of RNA and morphological analysis.

It is an object of the present invention to provide a general collection medium for identifying cellular morphology and quantitatively preserving DNA, RNA and other biomolecules from a single sample regardless of the disease to be detected.

It is another object of the present invention to provide a universal cell collection medium for preserving cell morphology and nucleic acids, lipids, carbohydrates, or proteins in a cell sample where limited numbers of cells are available in the sample.

It is another object of the present invention to provide compositions and methods for solution-based direct analysis of biomolecules of interest. The methods and compositions of this invention solve problems encountered in non-solution-based

methods such as *in situ* hybridization or non-direct methods which require separation of the biomolecule of interest from other cellular components before analysis.

SUMMARY OF THE INVENTION

The present invention relates to the detection, analysis and monitoring of cellular disease. A new cell collection medium is disclosed which preserves both cell morphology and cellular biomolecules for quantitative analysis in a cell sample so that multiple assays can be carried out from a single patient sample. The state of the cells can be assessed using a device for collecting cellular samples in a small volume.

One embodiment of the present invention involves examining the cell morphology and detecting a specific DNA sequence or measuring the levels of expression of genes involved in a cell state, and comparing their expression to each other or to reference genes in a specific ratio, as an indication of the state of a disease in the cells. This method can be used to detect and/or monitor the onset or progression of any human condition which causes a change in cell morphology or in levels or structures of specific biomolecules. For example, the present invention can be used to assess predisposition to a particular disease or to assess the stage or risk of a disease as indicated by the state of the cells. It can also be used to guide or assess the effectiveness of a therapy for a disease by identifying appropriate therapy based on the indicated cell state or by indicating any change in the state of cells subjected to the therapy.

In an other embodiment of the invention, a universal cell collection medium is disclosed. This medium allows simultaneous preservation of cell morphology and biomolecules in a small volume. Also embodied in the present invention is a device for collecting such cell samples.

In a further embodiment of the invention, methods and compositions are provided wherein a sample is analyzed according to cell morphology and biochemical analysis in solution phase. The biochemical analysis is either qualitative or quantitative and directly analyzes RNA, DNA, protein, carbohydrate or any

combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig 1. UCM 127, baseline (12 hours at RT). CaSki cells. Magnification x 200. Papanicolaou staining.

Fig 2. UCM 127, 3 weeks at RT. CaSki cells. Magnification x 200. Papanicolaou staining.

Fig 3. UCM 127, 6 weeks at RT. CaSki cells. Magnification x 200. H&E staining.

Fig 4. UCM 128, (12 hours at RT). CaSki cells. Magnification x 200. Papanicolaou staining.

Fig 5. UCM 128, 3 weeks at RT. CaSki cells. Magnification x 200. Papanicolaou staining.

Fig 6. UCM 128, 6 weeks at RT. CaSki cells. Magnification x 400. H&E staining

Fig 7. UCM 130,baseline (12 hours at RT). CaSki cells. Magnification x 200. H&E staining.

Fig 8. UCM 130, 3 weeks at RT. CaSki cells. Magnification x 200. Papanicolaou staining.

Fig 9. UCM 130, 6 weeks at RT. CaSki cells. Magnification x 400. H&E staining.

Fig 10. PreservCyt (134), baseline (12 hours at RT). CaSki cells. Magnification x 200. H&E staining.

Fig 11. PreservCyt (134), 3 weeks at RT. CaSki cells. Magnification x 200. H&E staining.

Fig 12. PreservCyt (134), 6 weeks at RT. CaSki cells. Magnification x 400. H&E staining.

Fig 13. CytoRich (135), baseline (12 hours at RT). CaSki cells. Magnification x 200. Papanicolaou staining.

Fig 14. CytoRich (135), 3 weeks at RT. CaSki cells. Magnification x 200. Papanicolaou staining.

Fig 15. CytoRich (135), 6 weeks at RT. CaSki cells. Magnification x 400. H&E staining.

Fig 16. UCM 141, 6 weeks at RT. CaSki cells. Magnification x 400. H&E staining.

Fig 17. UCM 149, 6 weeks at RT. CaSki cells. Magnification x 400. H&E staining.

Fig 18. UCM 141, baseline at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 19. UCM 141, baseline at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 20. UCM 141, 10 days at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 21. UCM 141, 10 days at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 22. UCM 141, 10 days at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 23. UCM 141, 10 days at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 24. UCM 149, baseline at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 25. UCM 149, baseline at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 26. UCM 149, 10 days at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 27. UCM 149, 10 days at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 28. UCM 149, 3 weeks at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 29. UCM 149, 3 weeks at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 30. PreservCyt, baseline at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 31. PreservCyt, baseline at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 32. PreservCyt, 10 days at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 33. PreservCyt, 10 days at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 34. PreservCyt, 3 weeks at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 35. PreservCyt, 3 weeks at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 36. CytoRich, baseline at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 37. CytoRich, baseline at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 38. CytoRich, 10 days at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 39. CytoRich, 10 days at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 40. CytoRich, 3 weeks at RT. Normal epithelial and CaSki cells. Magnification x 200.

Fig 41. CytoRich, 3 weeks at 4 °C. Normal epithelial and CaSki cells. Magnification x 200.

Fig 42. Conventional Pap smear (archival). Magnification x 120.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a universal cell collection medium that makes it possible to conveniently collect and preserve cells and their contents for assessment of the existence or progression of a disease isolated from a single small patient sample, using cytological assays, molecular assays, or both.

The instant universal cell collection medium preserves cell morphology and preserves macromolecules in a cell sample for either qualitative or quantitative analysis. One useful form of the disclosed cell collection medium preserves nucleic acids in the cells. Such preservation can be limited to refrigerated samples. Alternatively, preserved samples can be kept at ambient temperatures. Different forms of the universal collection medium preserve a sample for days or weeks or more. The universal cell collection medium can be used to collect cell samples for any purpose and is not limited to use with any particular assay method. Some forms of the universal collection medium contain a buffered saline isotonic solution or an alcoholic solution such as methanol, ethanol, or a similar alcohol, an RNase inhibitor such as RNasin, and a protease inhibitor such as pepstatin. Many different nuclease inhibitors are known in the art, including, for example, vanadate complexes, chelating agents and detergent-based compounds as well as specific inhibitors such as RNasin. Any known nuclease and/or protease inhibitors can be employed in the present invention as a component of the universal collection medium in order to preserve the particular

molecules of interest in the a sample.

The formulations of this invention provide for the first time means for performing cytological and molecular analysis on cells which are contained in a single sample. The cells are obtained from a patient and stored in the UCM of this invention. From this single sample, cells are extracted and a cytological examination is performed, the cellular DNA is qualitatively or quantitatively examined, the cellular RNA is qualitatively or quantitatively examined, or any combination of analysis is performed. The different analyses are performed concurrently or, for example, after the results of the cytological analysis are obtained, the cells are subjected to molecular analysis days, weeks or even months later. Conversely, after molecular analysis of the cells contained in the UCM, for example by automated screening, the sample is retrieved for cytological analysis, days, weeks or even months later.

In one embodiment of the invention the universal collection medium (UCM) formulations of this invention are buffered, water-based solutions which comprise a preservative such as a mixture of one or more alcohols, a cross-linking agent and an agent to inhibit degradation of RNA, DNA and protein. The use of the UCM formulation is further enhanced by the addition of an antimicrobial agent.

Nearly any non-viscous alcohol can be used to formulate the UCM, for example, any C1 to C10 alcohols or mixtures thereof can be used. Preferred alcohols include methanol, ethanol, propanols, butanols, and pentanols. Most preferred are ethanol and n-butanol. The alcohol can comprise a significant percentage of the formulation. For example, the alcohol(s) component can comprise about 1% to about 75% of the UCM formulation. More preferred is the percentage range of about 1% to about 50% alcohol and more preferred is about 5% to about 30% alcohol in the UCM formulation.

The pH range of the UCM formulation is important for maintaining the cellular biochemical and morphological integrity of the cells. A pH range of about 2.5 to about 6 is used to formulate the UCM of this invention. More preferred is a pH range of about 3 to about 5 and most preferred is a pH range of about 3.5 to about 4.5.

Buffer(s) are used to maintain the pH of the UCM at a constant value. Any buffer that has buffering capacity in the indicated pH range can be used in the UCM of this invention. Non-limiting examples of buffer components include glycine, maleic, phosphoric, tartaric, citric, formic, or acetic acids and the like.

The cross-linking agents of this invention comprise about 1% to about 25% of the UCM formulation. Preferably, the cross-linking agents comprise about 1% to about 15% or from about 1% to about 10%. Most preferably, the cross-linking agent comprises about 1% to about 5% of the UCM formulation. Cross-linking agents (also known as fixatives) are well-known in the art (*see*, for example, Stedman's Medical Dictionary, 25th Edition, Williams & Wilkins, Baltimore MD 1990 at page 592) and their use in the UCM formulation is now readily apparent to those of skill in the art in light of the present invention. Non-limiting examples of the cross-linking agents for use in the UCM formulation include aldehydes such formaldehyde, glutaraldehyde and the like. A preferred cross-linking agent is glutaraldehyde-bisulfite.

Agents able to inhibit degradation of RNA, DNA and/or protein are well-known in the art. They can work by either inhibiting enzymes or sequestering metal ions or both. Nuclease or protease inhibitors such as RNasin or pepstatin or chelating agents can be used according to this invention. Preferred agents to inhibit degradation of RNA, DNA and/or protein are chelating agents. Chelating agents are well-known in the art (*see*, for example, *Data For Biochemical Research*, Third Ed., Rex M. C. Dawson et al., Oxford University Press 1986, at chapter 17) and are known to both attenuate metal ion-induced and enzymatic degradation of biopolymers. Non-limiting examples of chelating agents for use in this invention include murexide, chromotropic acid, 1-(1-hydroxy-2-napththylazo-2-hydroxy-5-nitronaphthalene-4-sulphonic acid, EDTA (ethylenediaminetetraacetic acid), *o*-phenanthroline, thiourea and the like. A preferred chelating agent is EDTA.

Antimicrobial agents for use in this invention are those known in the art. Non-limiting examples of antimicrobial agents are aminoglycosides, ß-lactams,

cephalosporins, macrolides, penicillins, azides and the like. A preferred antimicrobial agent is sodium azide.

The universal collection medium can be used for a combination of two or more assays of different characteristics related to a cell state of interest. As used herein, the assay or assays refer to detection or measurement of specific characteristics, the results of which may be combined with other such measurements of other characteristics to provide an overall assessment of a cell suspected of being infected with one or more diseases. These assays may include, for example, a combination of morphological analysis and quanitation of a particular RNA or DNA or protein or carbohydrate structure whose presence or levels provide a specific indication of the presence or progression of a disease.

The universal collection medium can be used to collect any desired cell sample. Cell samples are collected in any suitable manner, including scrapings, biopsy, or washings, and from any suitable source. Numerous cell collection techniques are known and any can be used with the present invention. Generally, the source of cells for a cell sample is chosen based on the known or likely tissue affected by the cell state of interest.

Cell samples for use in the present invention can be collected and stored in liquid medium. Examples of useful cell collection media are PreservCyt® (Cytyc), and CytoRich™ (Autocyte). These media were developed for the collection of cytological samples but can be adapted for use with molecular assays when modified as described herein.

Nucleic acid detection generally benefits from the use of a reagent capable of preventing nucleic acid degradation prior to performing the assay if the assay is not performed soon after sample collection. A useful medium is a preservative based collection medium that has stabilizers for nucleic acids (both RNA and DNA) and proteins and that preserves cell morphology, such as the universal collection medium of the present invention.

One method useful with the present invention involves measuring the

levels of expression of genes involved in a disease state, and comparing their expression to each other or to reference genes, as an indication of the state of the cells. Such measurements can be combined with other assays to increase the accuracy and reliability of the assessment of the disease state. The present invention can be used to assess the stage of a disease as indicated by the state of the cells. This embodiment can also be used to guide or assess the effectiveness of a therapy for a disease by identifying appropriate therapy based on the indicated disease state or by indicating any change in the state of cells subjected to the therapy. Also disclosed is a cell collection medium for preserving cell morphology and cellular biomolecules in a cell sample so that multiple assays can be carried out on the same sample.

Many diseases and other human conditions are characterized by specific cellular phenotypes and gene expression patterns. Such diseases and conditions can be identified and/or monitored by assessment of specific cellular morphology or levels or structures of particular biomolecules. For example, neoplastic and cancerous cells generally exhibit certain distinctive morphologies and growth characteristics. Molecular characteristics, such as gene mutations and gene expression patterns are also a good indicator of disease progression. Virally infected cells can exhibit different morphologies and gene expression patterns, including expression of viral genes. Using the present invention, the characteristics of the cell state, such as changes in cell morphology and/or expression of genes can be determined from a patient sample.

The characteristics to be detected are specific to the cell state of interest and the disease suspected of being present in the cell sample. Such characteristics can be generally divided into two types, cytological characteristics and molecular characteristics. As used herein, cytological characteristics are characteristics such as, for example, overall cell shape and appearance of the cell and its organelles. The primary identification and classification of many neoplastic and cancerous cells has traditionally been accomplished using cytological characteristics. Identification of cytological characteristics is generally slow, requires a relatively high level of training,

and generally cannot be easily automated. As used herein, molecular characteristics are the presence and/or absence and state of particular molecular species, such as proteins, nucleic acids, carbohydrates and metabolites. Such molecular characteristics are generally identified by detecting and/or quantifying the particular molecules of interest.

The present invention allows both cytological and molecular characteristics to be analyzed from a small patient sample. The characteristics assayed can include additional or surrogate marker characteristics that are not a direct cause or result of the disease but that are related to certain disease and cell states. Examples of such additional markers include polymorphic markers, human leukocyte antigens (HLA) such as B7 that predispose women for cervical carcinomas, oncogenes, p53 mutations, BRCA1/2 mutations, other cancer markers, oncosuppressors, cytokines, growth factor receptors, and hormones. Such markers can be present in, or absent from, cells exhibiting state- or disease-specific characteristics, and such presence or absence can be indicative of, for example, a more severe or less severe disease state. These markers can be used in conjunction with disclosed methods to infer either higher or lower risk of neoplastic disease depending on the number of abnormal scores or the magnitude of change in quantitative markers.

Examples of disease states for assessment using the present invention include, but are not limited to, autoimmune disorders, neoplasias, and cancer. Other disease states of interest include HPV-based disease including HPV infection, cervical intraepithelial neoplasia (CIN), and cancer, atypical squamous cells of undetermined significance (ASCUS), warts, epidermo dysplasia verruciformis and other skin diseases, laryngeal papilloma, oral papilloma, conjunctival papilloma and prostate disease including enlarged prostate and prostate cancer, chlamydia, and viral infections such as HIV and herpes.

A cell sample as the term is used herein is primarily a collection of cells from a patient. One method of obtaining cells is through non-invasive means, which is defined herein as obtained without the puncturing of a patient. Examples of non-

invasive means are, for example, cell samples obtained from urine or a nasal, epithelial, cervical or other cell surface scrape. Other methods for obtaining a cell sample are by needle biopsy, or tissue biopsy. The cells are collected into volumes of less than 10 ml. More preferably, the cells are collected into volumes of less than about 5 ml, and most preferred the cells are collected into volumes of less than about 2 ml.

Combinations of multiple assays may be used with the media and device of the present invention and can be carried out from the collection and use of a single sample. An important aspect of the combination of assays is the use of a universal cell collection medium that allows a single cell sample to be used for multiple assays of different types with a minimum number of assay-specific processing steps required. For example, cells for assaying cytological characteristics are typically collected in a large volume of liquid which leaves the cell sample too dilute for most assays of molecular characteristics. Prior art methods do not provide for direct methods for both cytological and molecular analysis. The prior art methods require several extra steps, such as a separate concentration step, which is inconvenient and may result in many of the molecular components of the cell being degraded. Cytological cell samples, while optimized for preserving cell morphology, generally do not preserve nucleic acids well; thus making the sample unusable for many molecular assays.

Cell samples for use in the method of the present invention can be fixed or processed in any manner consistent with the assays to be performed. For example, both cytological and molecular assays can be performed using cells fixed on a solid substrate such as a slide. Preferably, however, molecular assays are done in solution. The requirements of the assays to be performed will generally determine the sample processing to be used.

The types of comparisons described above can also be used with many different genes and disease states. That is, the measured level of expression of a gene of interest can be compared, for example, to the level of expression of the same type of

gene in a different cell sample (such as an earlier cell sample from the same source or appropriate reference cells), to the level of expression of a different type of gene in the same or a different cell sample, to the level of expression of a reference gene in the same cell sample, or to the level of expression of a reference gene in reference cells.

Expression of genes of interest can be assessed using any suitable method. For example, RNA can be detected using hybridization, amplification, or sequencing techniques, and protein, like carbohydrate, can be detected using specific antibodies. Many techniques for the specific detection of gene expression, by detection of expression products, are known and can be used with the disclosed UCM. One technique for detecting and measuring the level of expression of genes of interest is detection of RNA transcribed from the genes of interest. For the most reliable comparisons, expression levels that are to be compared should be measured using the same technique and be performed in the same manner.

Useful techniques for measuring the level of expression of a gene of interest in a cell sample include the hybrid capture technique described in WO 93/10263 by Digene, PCR in situ hybridization techniques described by (Nuovo, 1997)), branched DNA assays (Chernoff (1997)), transcription mediated amplification (TMA); Stoflet (1988)), and polymerase chain reaction (PCR), ligase chain reaction (LCR), self-sustained sequence replication (3SR), nucleic acid sequence based amplification (NASBA), strand displacement amplification (SDA), and amplification with Qß replicase (Birkenmeyer and Mushahwar, (1991); Landegren, (1993)). Numerous assays for the detection and measurement of gene expression products are known and can be adapted for the determination of the level of expression of genes of interest using the UCM-collected samples.

The present invention provides methods for analyzing cells that previously were unavailable. For example, a combination of cancerous and non-cancerous cells were placed in a Universal Collection Medium according to this invention. The morphology of the cells was examined after storage at either 4 °C or room temperature for periods of 12 hours, 10 days, 3 weeks and 6 weeks. Slides were

prepared by staining using procedures well-known in the art, for example, Papanicolaou staining and Hematoxylin and Eosin (H&E). When the slides were examined under a light microscope, all the features characteristic for cancer cells were visible and well-preserved in all cases. The slides showed large and hyperchromatic nuclei, usually with oval shape and irregular, granular chromatin, scanty cytoplasm, multinucleated cells, presence of nucleoli and presence of mitotic figures. In addition, the slides showed that there was good cell dispersion and no cell clumping. Both types of cells (normal and cancerous) had distinct and sharp shapes of both the nuclei and cytoplasm. The nuclei were well-stained and the normal cells showed a different color of cytoplasm depending on the maturity of the cell.

In one embodiment of the present invention RNA was analyzed directly by solution based procedures. The cells were first lysed by adding a proteolytic enzyme to the cells contained in wells of a microtiter plate. Non-limiting examples of enzymes for use in the present invention include proteinase K or Pronase. Cells can also be subjected to detergent lysis or osmotic lysis or a French Press. After incubation, biotinylated DNA probes were added to each well. The RNA:DNA hybrids were captured onto a solid phase by transferring to streptavidin coated microplates. Alkaline phosphatase-conjugated antibodies to RNA:DNA hybrids were added to each well in the hybridization microplate and signals were generated by adding a chemiluminescent reagent such as CDP-Star™ with Emerald II (Tropix) to each well. The signal was read from the microplate. The solution based DNA analysis was performed similarly to the RNA analysis except that the microtiter plates were coated with anti-RNA:DNA hybrid antibodies and the probes were unlabeled RNA probes.

The present invention can be conveniently performed using kits that include one or more of the materials needed for the method, such as reagents and sample collection and handling materials. For example, kits can include cell collection medium including sample preserving reagents, reagents for specific detection of DNA sequences, RNA sequences and/or expression products (mRNA or protein) of one or

more DNA sequences, and sample handling containers. Useful reagents for detection of DNA sequences and/or RNA sequences are nucleic acid probes or protein nucleic acid probes for those sequences. Useful reagents for detection of DNA sequence expression products (proteins) are antibodies. Aberrant carbohydrate antigens associated with cancerous cells can also be detected by antibodies. A kit may also contain control samples or reagents, or reagents and materials for performing other assays to be combined with the disclosed assay.

The present invention can be performed using devices adapted to the method. Numerous devices for performing similar assays are known and in use and can be adapted for use with the disclosed UCM formulations, assays and methods. For example, devices are known for automating all or a part of sample assays and sample handling in assays.

All or part of the disclosed method can be controlled or managed using special purpose computer programs. The data collected from the disclosed method, and data from any other assay used in combination, can be compiled, analyzed, and output in various forms and for various purposes using special purpose computer programs. Such programs can be used with, or combined into, other patient or data management computer programs. The usefulness of such a program increases with the number of measurements or assessments to be combined, and the relative importance of each type of measurement to the overall assessment. Computer programs for use with the disclosed method can be used on general purpose computers, or can be incorporated into special purpose computers or computerized devices for controlling the disclosed method, handling and analyzing data from the disclosed method, or both.

EXAMPLES

The examples herein are meant to exemplify the various aspects of carrying out the invention and are not intended to limit the invention in any way.

Formulations

Formulation 127

20% Ethanol
0.05% NaN_3
5 mM EDTA
2.5% Glutaraldehyde-Sodium-Bisulfite (G-S-B)
0.2 M NaOAc-HOAc
pH 3.7

Formulation 128

20% Ethanol
0.05% NaN_3
5 mM EDTA
2.5% Glutaraldehyde-Sodium-Bisulfite (G-S-B)
0.2 M NaOAc-HOAc
pH 4.1

Formulation 130

7% Butanol
0.05% NaN_3
5 mM EDTA
2.5% Glutaraldehyde-Sodium-Bisulfite (G-S-B)
0.2 M NaOAc-HOAc
pH 3.7

Formulation 134

PreservCyt® (Cytyc Corporation)
Contains buffered methanol
Storage limits with cytologic samples: 3 weeks at 4 °C - 37 °C

Formulation 135

CytoRich™ (AutoCyte Corporation)
Contains less than 24% alcohol
Storage 15 °C - 30 °C

Formulation 141

20% Ethanol
0.05% NaN_3
5 mM EDTA
2.5% Glutaraldehyde-Sodium-Bisulfite (G-S-B)
0.2 M NaOAc-HOAc
pH 4.4

Formulation 149

10% Butanol
0.05% NaN_3
5 mM EDTA
2.5% Glutaraldehyde-Sodium-Bisulfite (G-S-B)
0.2 M NaOAc-HOAc
pH 4.4

Example 1: General Methods for Nucleic Acid Analysis

The assay for nucleic acids follows in general principle the method for detecting HIV RNA by the Digene Hybrid Capture HIV Test, described in WO 93/10263 by Digene. Briefly, following lysis, 50 µl of probe mix (containing DNA biotinylated probe) was added to each well. The plate was sealed and incubated at 65 °C for 1.5 hours for hybridization to occur. After hybridization, samples were transferred to a strepavidin-coated microplate, and 25 µL of anti-hybrid antibody was added to each well. The plate was agitated at 1100 RPM, for 1 hour, at room temperature. Wells were washed 6X times with 65 °C wash buffer, followed by one wash using distilled water. 100 µl of a chemiluminescent substrate was added to each well and the plate was incubated at room temperature for 30 minutes. The plate was then read in the DML 2000 luminometer. The data was then expressed as signal-to-noise. Using a calibration curve, the chemiluminescent signal generated by each specimen was converted into mRNA copies per cell.

Example 2: General Methods for Morphological Analysis

HPV 16 positive cancer cells (CaSki) were placed in UCM 127, 128, 141, 130, 149 and in two commercially available fixatives PreservCyt (Cytyc Corp.) and CytoRich (AutoCyte). The samples were then stored at ambient temperature. The baseline slides were prepared after 12 hours of storage. Then slides were prepared after 3 and 6 weeks. In addition slides were prepared from UCM 141 and 149 after 6 weeks at RT. The slides were prepared by spotting 200 µl of cell suspension onto polycarbonate filter. The filter was then placed on the glass slide and blotted. The filter was then removed and the slides fixed in 95% ethanol for 5 minutes. The slides were stained using routine Papanicolaou staining and Hematoxylin and Eosin (H&E). The slides were evaluated under a light microscope using different magnifications, and documentation in the form of color pictures was prepared. Additionally a smaller study was performed using a mixture of normal human cervical cells and CaSki cells. This mixture was placed in two UCM formulations 141 and 149 and in PreservCyt and CytoRich controls. Each sample was split into two tubes and placed at RT and at 4 °C. After 12 hours (baseline), 10 days and three weeks of storage, slides were prepared and stained with Papanicolaou staining. The following features were evaluated for the morphology study: cell dispersion, cell shape, nuclear shape, chromatin pattern and staining intensity, nuclear/cytoplasm ratio, presence of nucleoli, cytoplasm shape and staining color. Figures 1–24 show the morphology of CaSki cells stored in different UCM and in PreservCyt and CytoRich controls stored at RT for 12 hours, 3 weeks and 6 weeks. All features characteristic for CaSki cells (carcinoma cells) were visible and well preserved in all fixatives tested after 3 and 6 weeks: large and hyperchromatic nuclei usually with oval shape and irregular granular chromatin, scanty cytoplasm, multinucleated cells, presence of nucleoli and presence of mitotic figures. In addition, the slides showed that there was good cell dispersion and no cell clumping in all media tested.

Figures 25-72 show the morphology of normal human cervical cells mixed with CaSki cells and stored in UCM 141, 149 and PreservCyt and CytoRich

controls for 12 hours, 10 days and 3 weeks at room temperature and at 4 °C. The evaluation of these slides showed that the morphology was well preserved in UCM and in PreservCyt and CytoRich controls. After 3 weeks at 4 °C and room temperature both types of cells (normal cervical and CaSki cells) had distinct and sharp shapes (both nuclei and cytoplasm). The nuclei were well stained and the normal cells showed a different color of cytoplasm depending on the maturity.

Figures 73-75 were taken from archived routine Pap smears to show the morphology and staining of samples stored fixed to slides for comparison.

Example 3: HC II HPV DNA Assay Results

UCM formulations 127, 128, 130 and the STM™ (Digene) control were tested using the Hybrid Capture II HPV DNA Test. A standard HC II HPV Test kit (Digene catalog number 5101-1096) was used. Each collection medium (1 mL) was spiked with 0.8 x 10^6 CaSki cells (~500 copies/cell). This concentration of CaSki cells was chosen because an adequate clinical specimen usually contains about 1 x 10^6 cells. The same stock of CaSki cells was then used for morphology study and DNA and RNA testing. A standard volume of 50 µl was used per assay as described in the Package Insert, without any sample preparation modification. A similar STM sample was prepared by spiking the same number of CaSki cells into 1 mL of Digene Sample Transport Medium (STM – this medium is the current medium used for HPV testing. It preserves DNA and RNA but not cell morphology). The HC II HPV test was performed at day "0" (baseline) and after one and six weeks of storage at room temperature. Table 1 shows the results obtained for each medium at the baseline and after one and six weeks expressed as S/N ratios.

UCM formulations 127 and 128 had the highest S/N ratios comparable to the STM control S/N ratios at the baseline and retained these values after storage at RT for six weeks. The S/N ratios for UM 130 obtained at the baseline were slightly lower when compared to UCM 127 and 128. UCM 130 retained its signal after 6 weeks.

Table 1. Signal to Noise ratios obtained for UCM 127, 128, 130 and STM at baseline and after one week and six weeks storage at RT.

Medium	Baseline S/N	Week 1		Week 6	
		S/N	%of original signal	S/N	%of original signal
127	5666	5852	103	6298	111
128	5760	5669	98	5932	103
130	4407	5135	117	5500	125
STM	5557	6151	110	6127	110

Formulations UCM 141 and 149, used in the smaller morphology study, were tested in the HC II HPV DNA assay at baseline and then after one, two and four weeks of storage at room temperature. CaSki cells (1.5×10^6) were spiked in these media and 50 µl were used per assay. Table 2 shows the S/N ratios. Both formulations retained close to 100% (UCM 141 98.4% and UCM 149 97.3%) of the original signal after four weeks of storage at room temperature.

Table 2. S/N ratios obtained for formulations UC 141 and 149 at baseline and after 1, 2 and 4 weeks of storage at RT.

UCM	Baseline S/N	Week 1		Week 2		Week 4	
		S/N	% original signal	S/N	% original signal	S/N	% original signal
141	10875	11006	101.2	10427	95.9	10703	98.4
149	9443	8159	86.4	8035	85.3	9188	97.3

In addition, samples stored in PreservCyt and CytoRich were examined according to the protocols of this example. Both the PreservCyt and CytoRich media were found to be deficient in their ability to provide DNA for satisfactory analysis.

Example 4: HC II RNA Assay Results

The aliquots of all formulations (CaSki cells 0.8×10^6) were stored at 4 °C instead of room temperature and were tested in the HC II HPV RNA assay. The prototype RNA assay used in this study utilized biotinylated single-stranded DNA probes specific for HPV 16 E6/E7 RNA. UCM formulations 127, 128 and 130 were

tested at baseline and after one and six weeks storage at 4 °C. UCM formulations 141 and 149 were tested at baseline and after one, two and four weeks of storage at 4 °C. Tables 3 and 4 show the results.

Table 3. Results obtained for UCM 127, 128 and 130 in HC II RNA assay at baseline and after one and six weeks of storage at 4 °C.

Medium	Baseline S/N	Week 1		Week 6	
		S/N	% original signal	S/N	% original signal
127	937	1157	123	990	106
128	981	1139	116	1192	122
130	845	1261	149	1278	151

All UCM formulations tested had comparable S/N ratios when tested at the baseline. UCM 127, 128 and 130 retained a 100% signal after six weeks of storage at 4 °C in comparison to baseline results. RNA in cells in both UCM formulations 141 and 149 appears to be stable at 4 °C for four weeks giving S/N ratios close to the baseline results (UCM 141 108% and UCM 149 89%).

Table 4. Results obtained for UCM 141 and 149 in HC II RNA assay at baseline and after one, two and four weeks of storage at 4 °C.

UCM	Baseline S/N	Week 1		Week 2		Week 4	
		S/N	% original signal	S/N	% original signal	S/N	% original signal
141	1074.5	1029	96	1137	106	1164	108
149	1243.5	1786	144	1843	148	1103	89

In addition, samples stored in PreservCyt and CytoRich were examined according to the protocols of this example. Both the PreservCyt and CytoRich media were found to be deficient in their ability to provide RNA for satisfactory analysis.

Publications cited herein and the material for which they are cited are specifically incorporated by reference.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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WE CLAIM

1. A cell or tissue collection medium (universal collection medium), wherein the cells or tissue contained in the medium can be analyzed directly by both cytological and molecular methods, wherein the molecular method of analysis comprises either RNA or DNA or protein analysis or the analysis of both RNA and DNA, and wherein the medium is water based and comprises a preservative, a cross-linking agent and an anti-degradation agent.

2. The universal collection medium of claim 1, wherein the medium consists of a volume of less than 10 ml.

3. The universal collection medium of claim 1, wherein the medium consists of a volume of less than about 5 ml.

4. The universal collection medium of claim 1, where in the medium consists of a volume of less than about 2 ml.

5. The universal collection medium of claim 1 wherein the universal collection medium comprises a buffer component, at least one alcohol component, a cross-linking agent and an agent to inhibit degradation of at least one of the group consisting of RNA, DNA and protein.

6. The universal collection medium of claim 5, wherein the buffer component has a buffering capacity within a pH range of about 2.5 to about 6.

7. The universal collection medium of claim 6, wherein the buffer component has a buffering capacity within a pH range of about 3 to about 5.

8. The universal collection medium of claim 7, wherein the buffer component has a buffering capacity within a pH range of about 3.5 to about 4.5.

9. The universal collection medium of claim 5, wherein the alcohol component comprises a C1 to C10 alcohol.

10. The universal collection medium of claim 9, wherein the alcohol component is selected from the group consisting of methanol, ethanol, propanols,

butanols, and pentanols.

11. The universal collection medium of claim 10, wherein the alcohol component comprises ethanol or n-butanol.

12. The universal collection medium of claim 5, wherein the cross-linking agent comprises an aldehyde.

13. The universal collection medium of claim 12, wherein the cross-linking agent is selected from the group consisting of formaldehyde and glutaraldehyde.

14. The universal collection medium of claim 12, wherein the cross-linking agent comprises glutaraldehyde-bisulfite.

15. The universal collection medium of claim 5, wherein the agent to inhibit degradation of at least one of the group consisting of RNA, DNA and protein comprises at least one agent selected from the group consisting of a nuclease inhibitor, a protease inhibitor and a chelating agent.

16. The universal collection medium of claim 15, wherein the agent to inhibit degradation of at least one of the group consisting of RNA, DNA and protein comprises a chelating agent.

17. The universal collection medium of claim 15, wherein the chelating agent is selected from the group consisting of murexide, chromotropic acid, 1-(1-hydroxy-2-napththylazo-2-hydroxy-5-nitronaphthalene-4-sulphonic acid, EDTA (ethylenediaminetetraacetic acid), *o*-phenanthroline and thiourea.

18. The universal collection medium of claim 12, wherein the chelating agent comprises EDTA (ethylenediaminetetraacetic acid).

19. A method of performing morphological and biochemical analysis on a cell or tissue, wherein the method comprises:

obtaining cells or tissues from a patient;

preserving the cells or tissue in a water-based medium comprising a preservative, a cross-linking agent and an anti-degradation agent;

directly analyzing the morphology of the cells or tissue preserved in the medium; and

directly analyzing either RNA or DNA or protein contained in the cells or tissue preserved in the medium.

20. A universal collection medium comprising water, a preserving agent, a buffer, a cross-linking agent and an agent capable of inhibiting the degradation of at least one of the group consisting of RNA, DNA and protein.

21. An article of manufacture for preserving a cell sample of limited cell number comprising:

a container holding less than 2 ml of the medium according to claim 20; and

a lid fitting said container.

22. The article of manufacture of claim 21 further comprising a cell collecting device having an elongated member wherein a distal end of the elongated member has a non-absorbent increased surface area.

23. The article of manufacture of claim 22 wherein the distal end of the elongated member is a brush.

24. A method of cell sample collection that allows detection of cell morphology and quantitative analysis of at least one of the group consisting of RNA, DNA and protein from a single sample, said method comprising

collecting cells from a patient wherein the cell sample is limited in size;

storing collected cells in the medium according to claim 20;

removing an aliquot of cells in the medium for cell morphology analysis; and

removing a second aliquot of cells in the medium for a quantitative

analysis selected from the group consisting of DNA analysis, RNA analysis, protein analysis and carbohydrate analysis.

25. The method of claim 24, wherein the cells are stored in a sample of less than 10 ml.

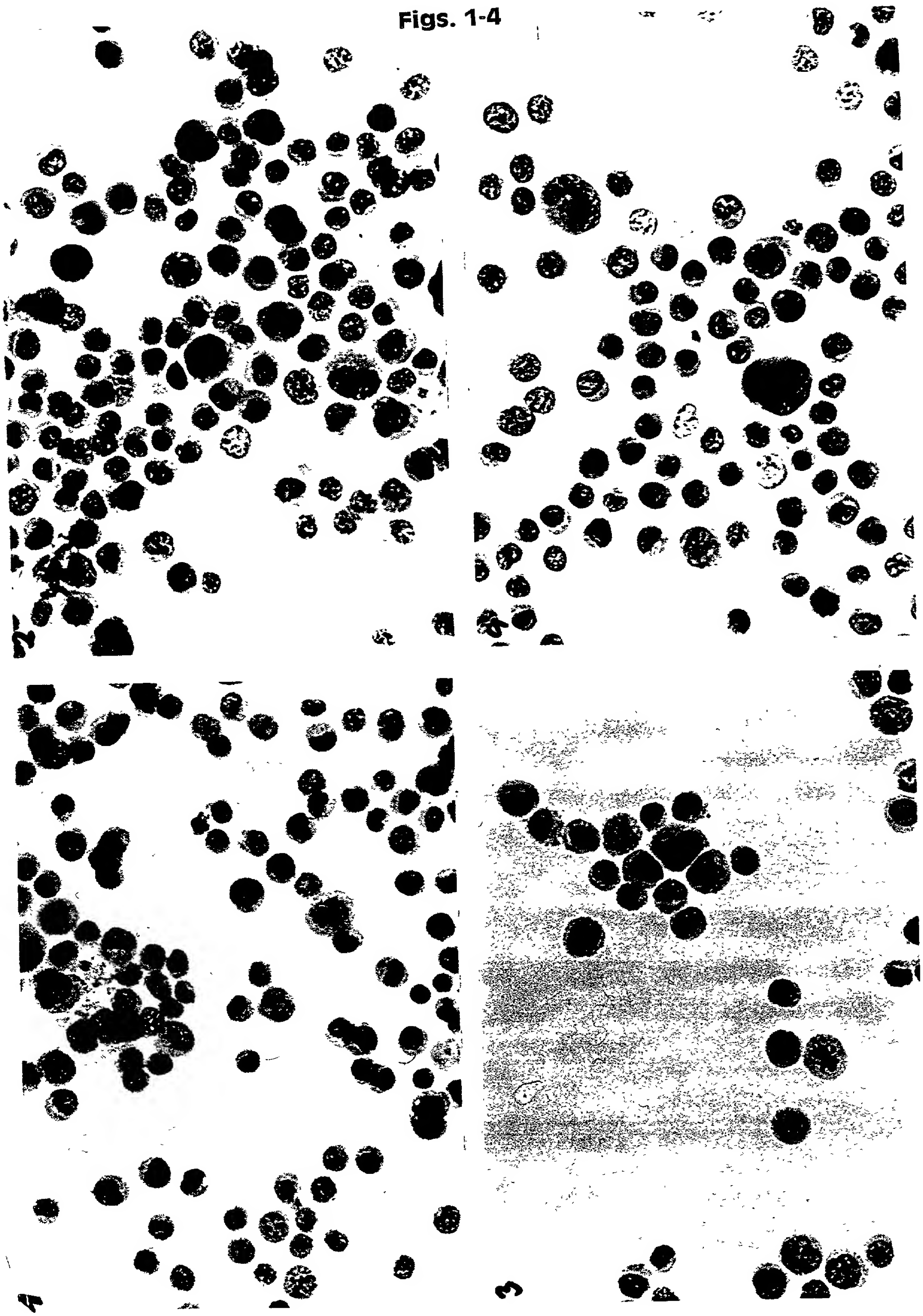
26. The method of claim 24, wherein the method of claim 24, wherein the cells are stored in a sample of less than about 5 ml.

27. The method of claim 24, wherein the cells are stored in a sample of less than about 2 ml.

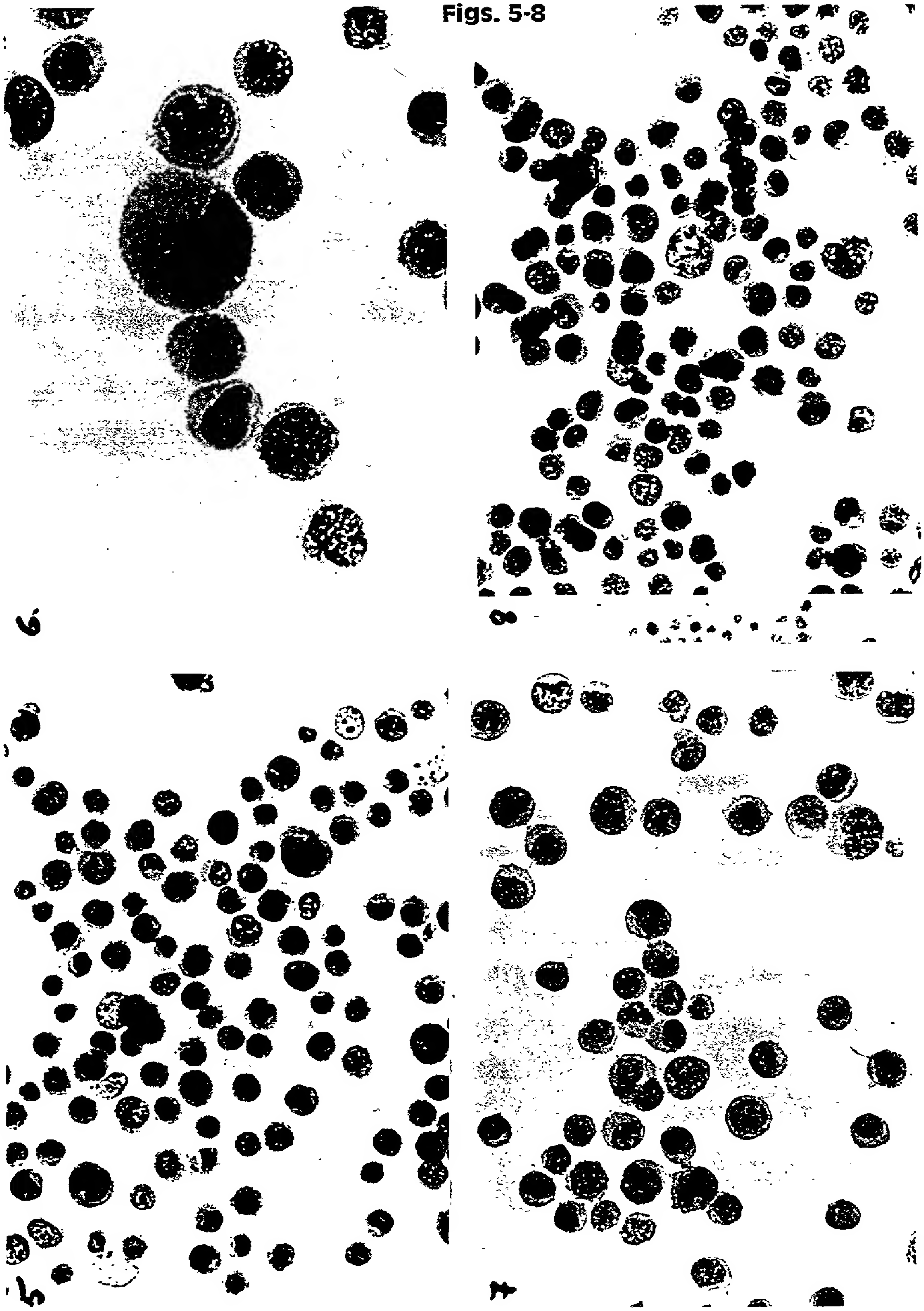
ABSTRACT

This invention provides a novel universal collection medium for cell collection. The medium allows for the first time the ability to perform cytology and direct molecular analysis on cells preserved in a single sample. This invention also provides novel methods for analyzing cells to assess human conditions.

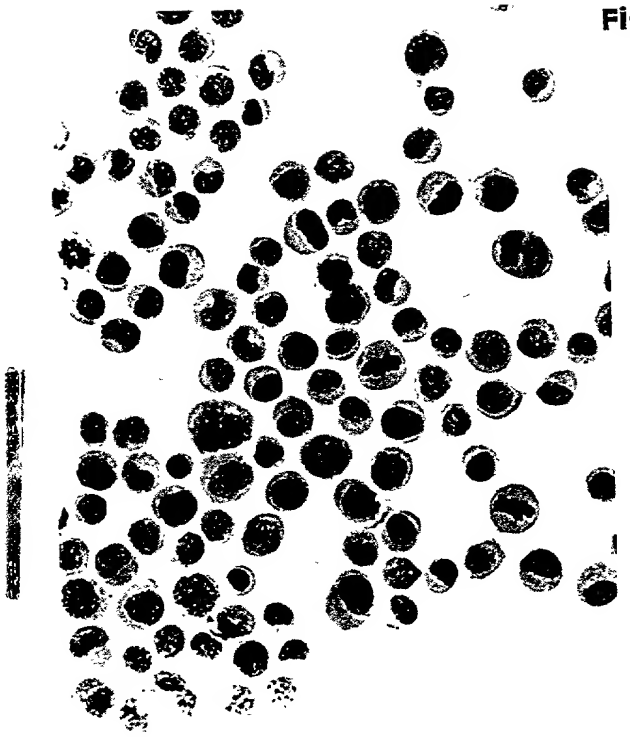
Figs. 1-4



Figs. 5-8



Figs. 9-12



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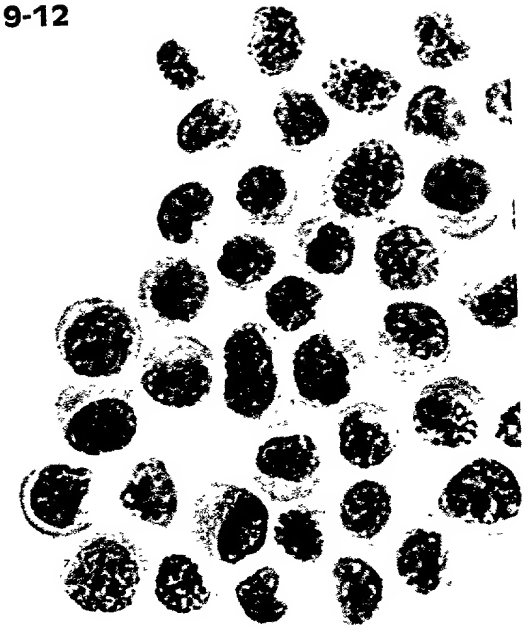

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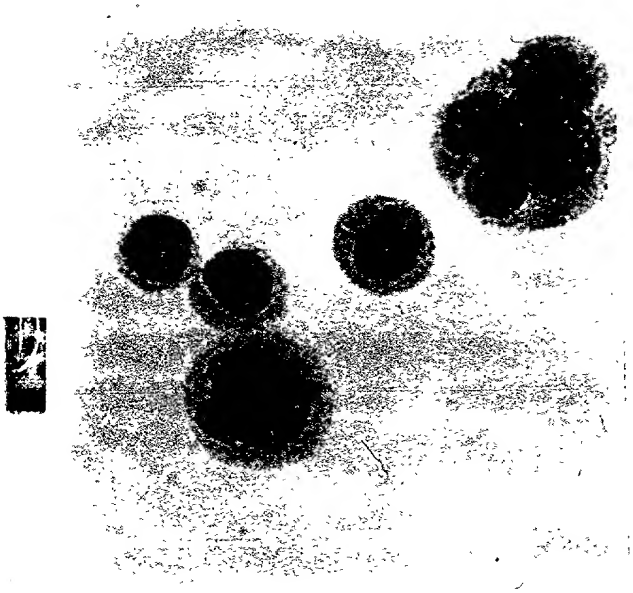

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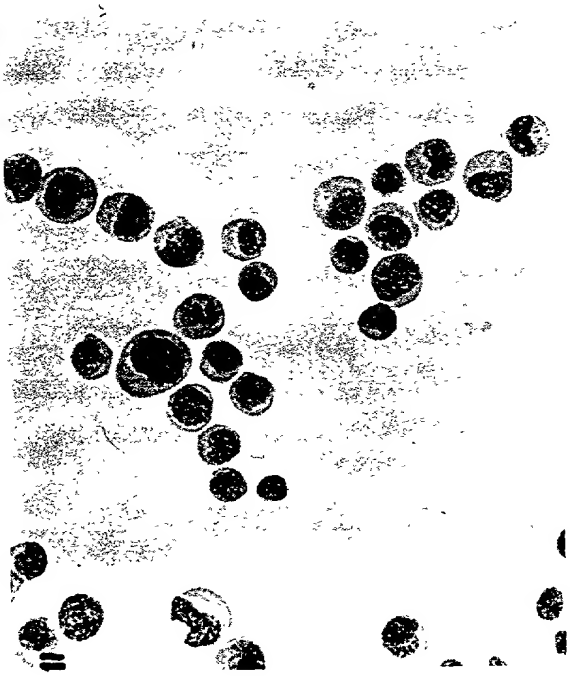

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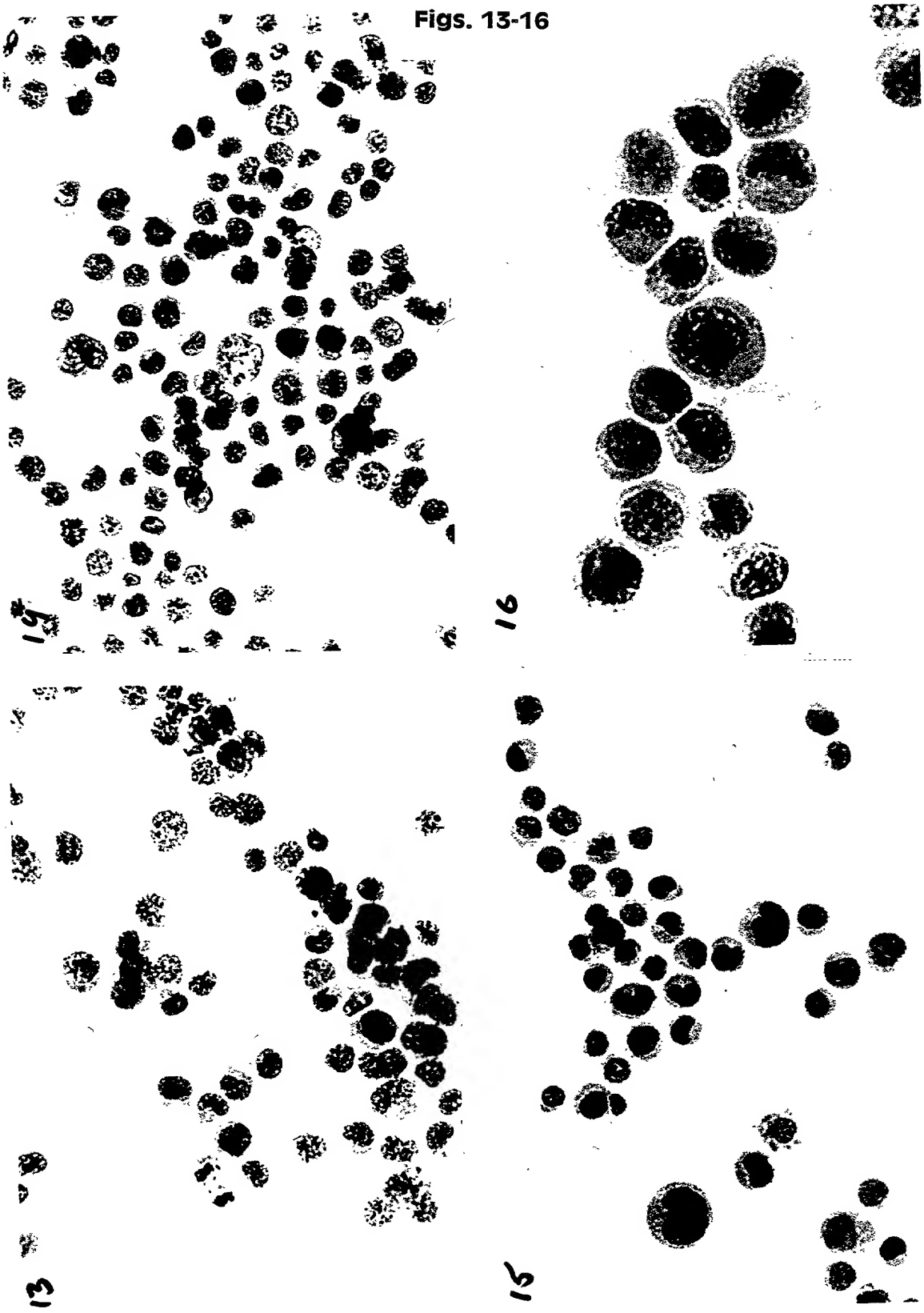
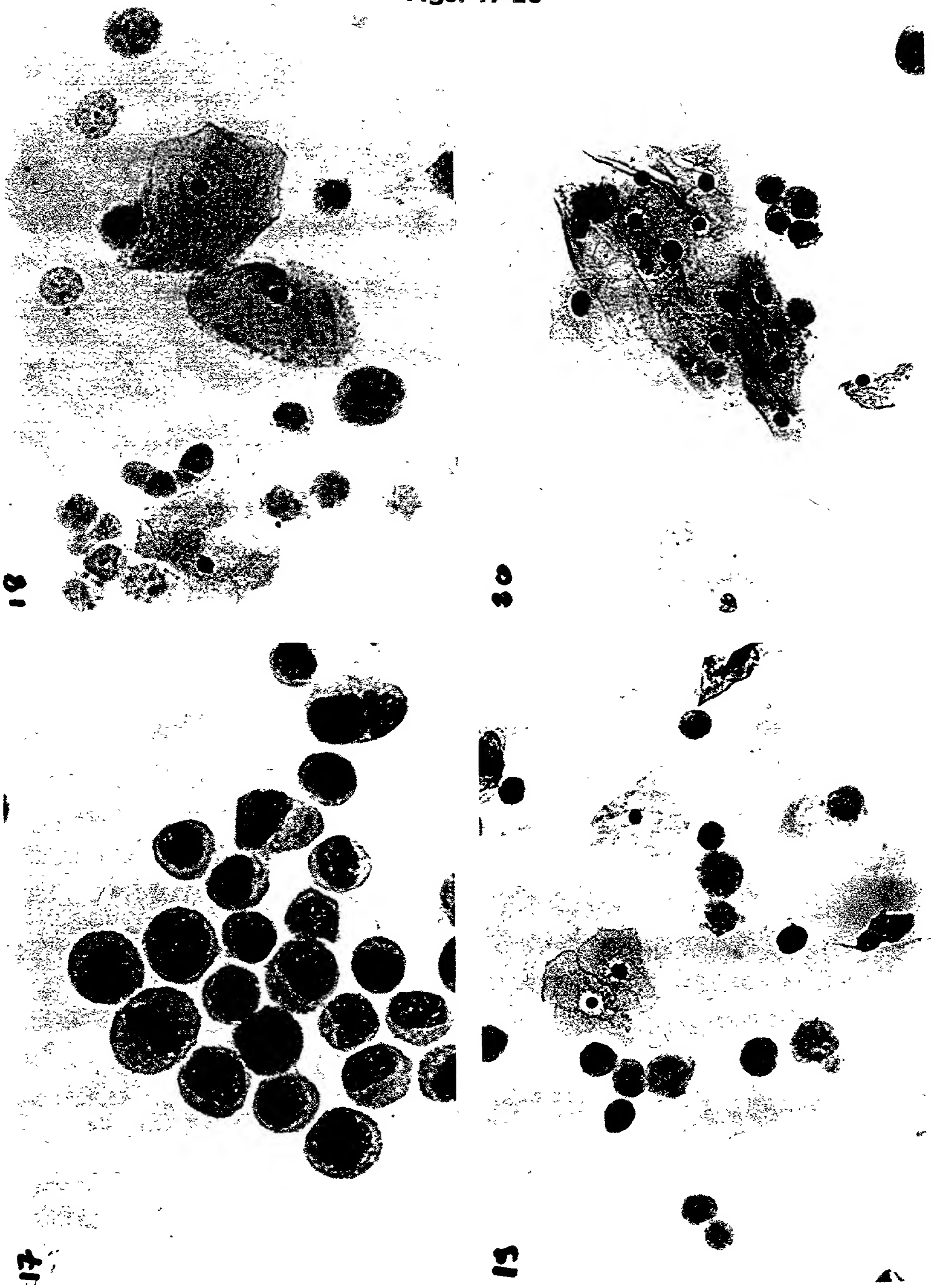
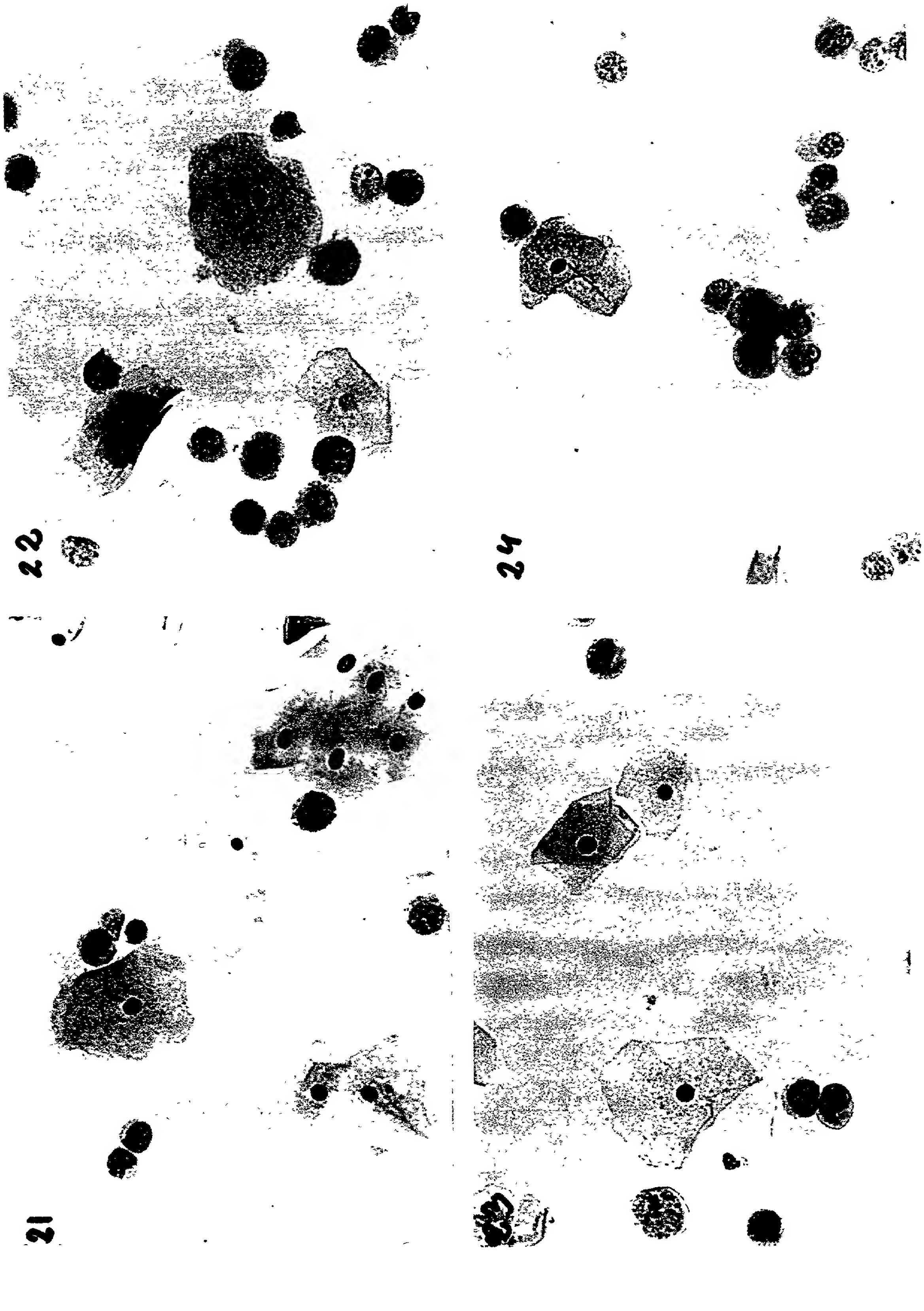
Figs. 13-16



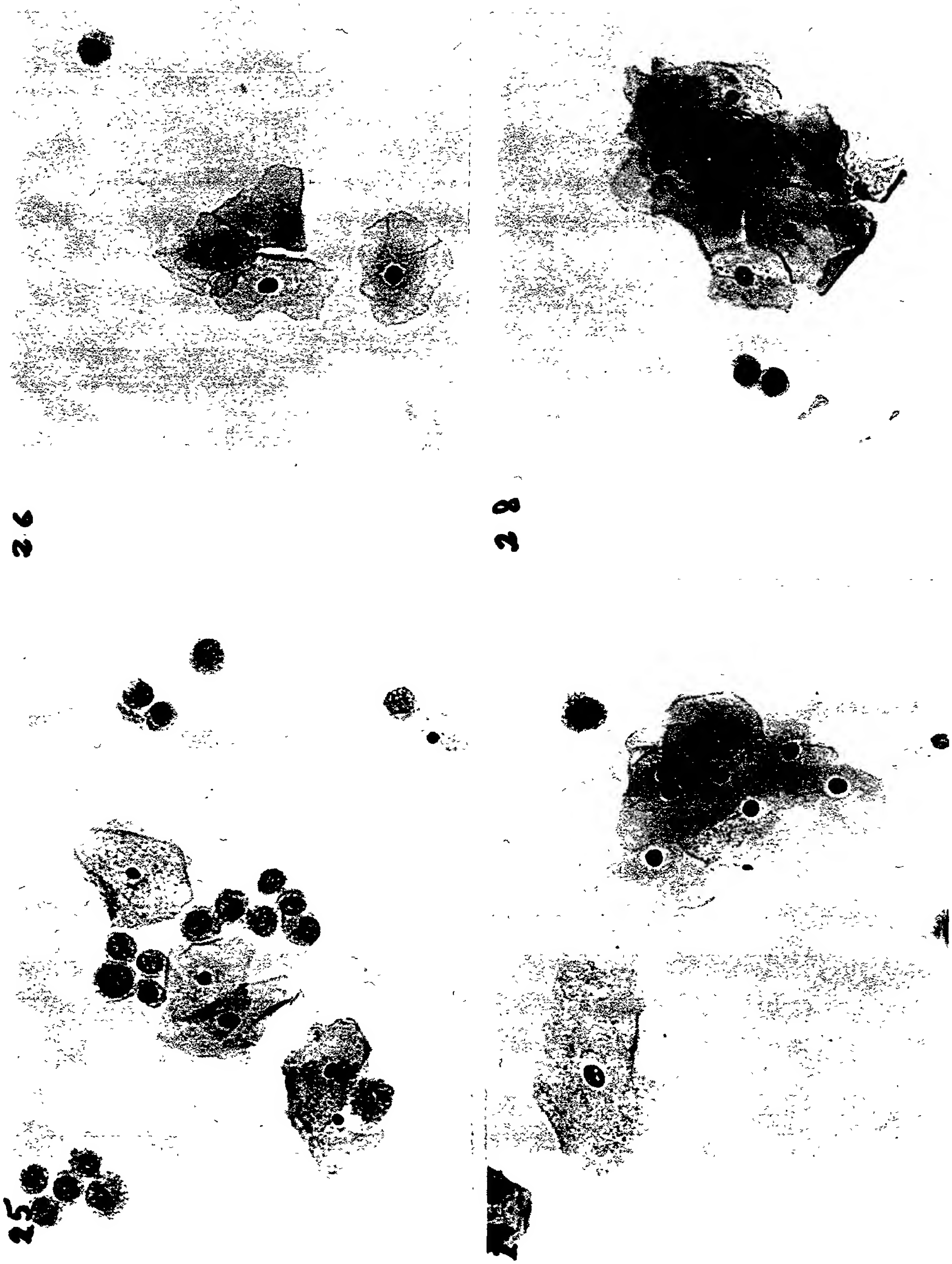
Figs. 17-20



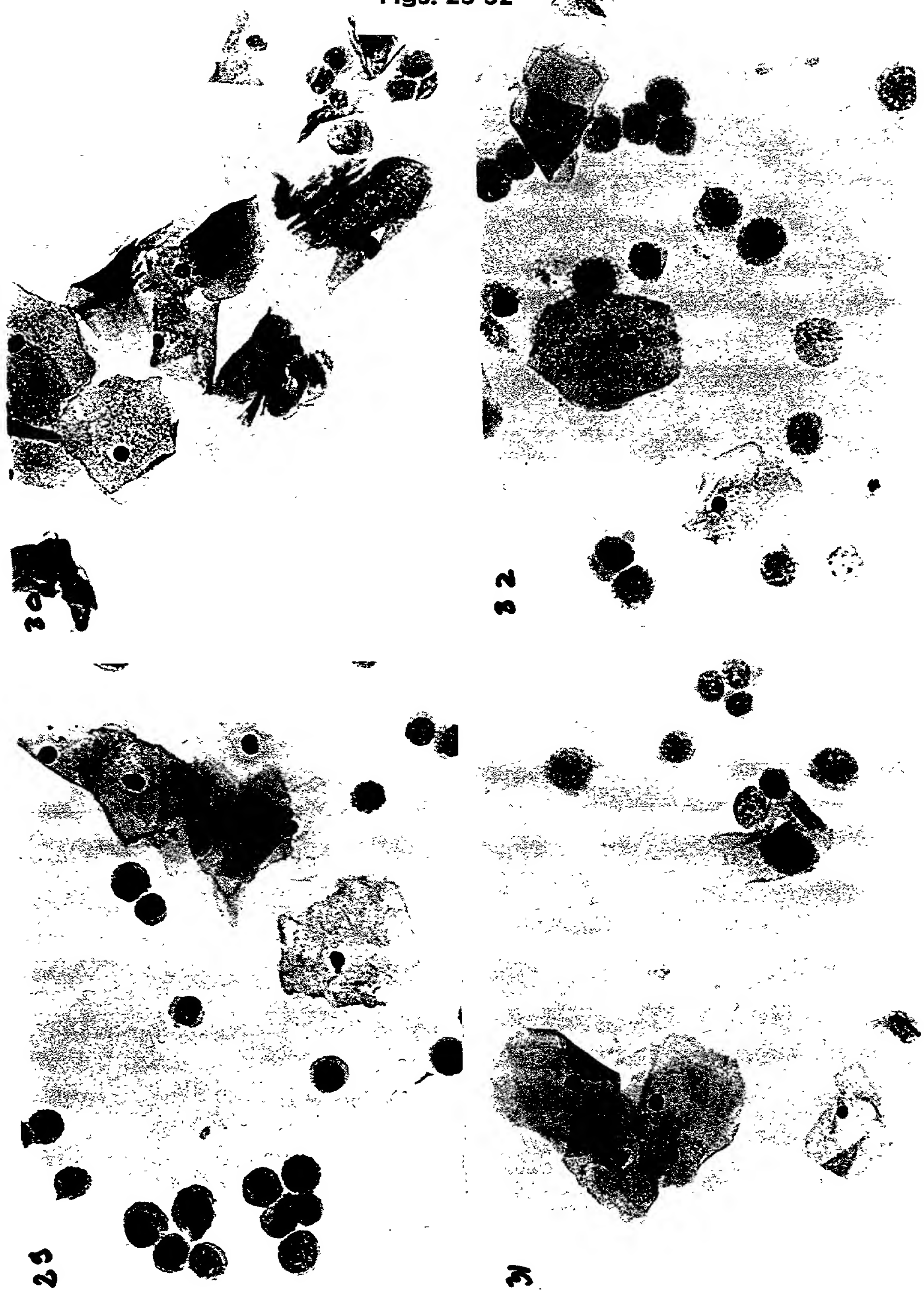
Figs. 21-24



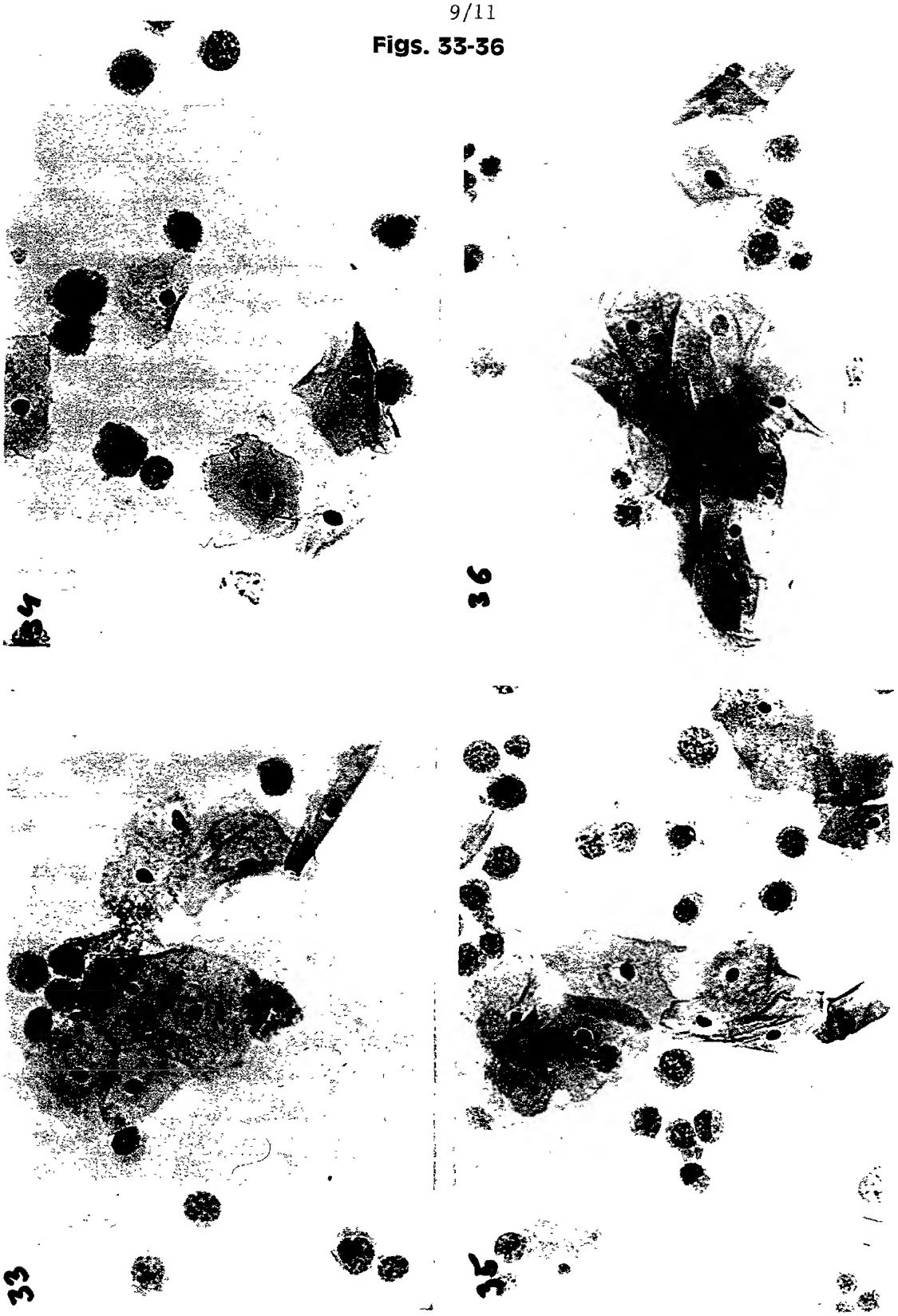
Figs. 25-28



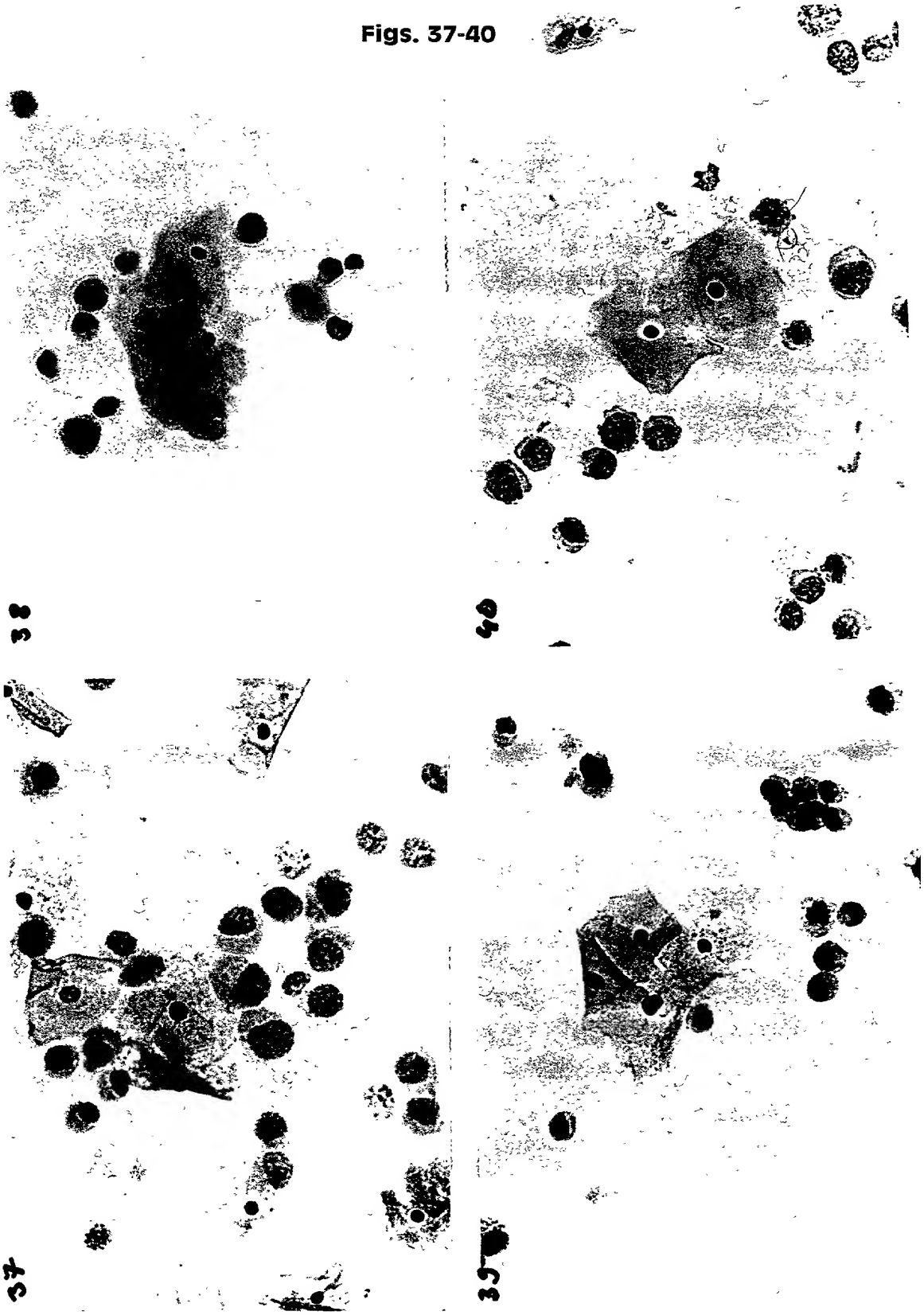
Figs. 29-32



Figs. 33-36



Figs. 37-40



Figs. 41-42

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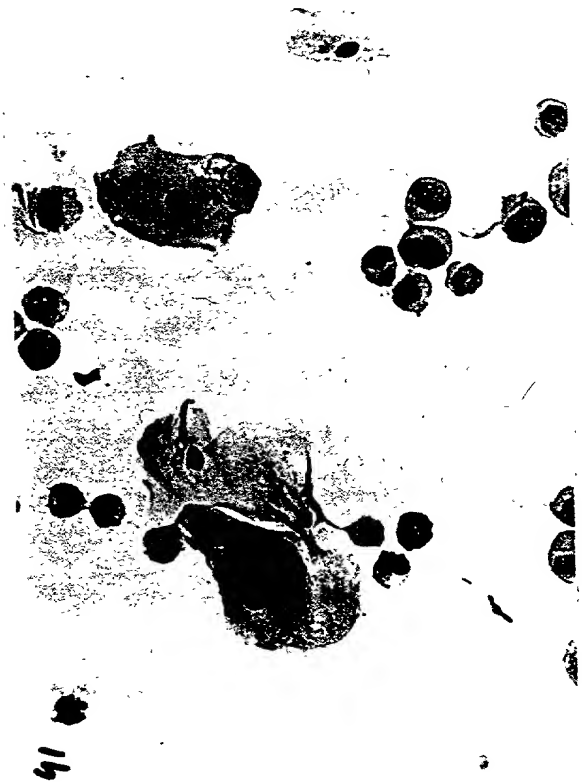

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Docket No. 2629-4005US1

COMBINED DECLARATION AND POWER OF ATTORNEY FOR
ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL
DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART APPLICATION

As a below name inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

UNIVERSAL COLLECTION MEDIUM
the specification of which

a. [X] is attached hereto

b. [ ] was filed on _____ as application Serial No. _____ and was amended on _____. (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STATE

c. [ ] was described and claimed in International Application No. _____ filed on _____ and as amended on _____. (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby specify the following as the correspondence address to which all communications about this application are to be directed:

SEND CORRESPONDENCE TO: MORGAN & FINNEGAN, L.L.P
345 Park Avenue
New York, N.Y. 10154

DIRECT TELEPHONE CALLS TO: Dorothy Auth
(212) 758-4800

[ ] I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) or under § 365(b) of any foreign application(s) for patent or inventor's certificate or under § 365(a) of any PCT international application(s) designating at least one country other than the U.S. listed below and also have identified below such foreign application(s) for patent or inventor's certificate or such PCT international application(s) filed by me on the same subject matter having a filing date within twelve (12) months before that of the application on which priority is claimed:

[ ] The attached 35 U.S.C. § 119 claim for priority for the application(s) listed below forms a part of this declaration.

Country/PCT	Application Number	Date of filing (day, month, yr)	Date of Issue (day, month, yr)	Priority Claimed
				[ ] YES [ ] NO
				[ ] YES [ ] NO
				[ ] YES [ ] NO

[X] I hereby claim the benefit under 35 U.S.C. § 119(e) of any U.S. provisional application(s) listed below.

Provisional Application No.	Date of Filing (day, month, yr)
60/082,167	17 April 1998
60/070,486	05 January 1998
60/069,426	12 December 1997

ADDITIONAL STATEMENTS FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART OR PCT INTERNATIONAL APPLICATION(S) (DESIGNATING THE U.S.)

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or under § 365(c) of any PCT international application(s) designating the U.S. listed below.

US/PCT Application Serial No.	Filing Date	Status (patented, pending, abandoned)/ U.S. application no. assigned (For PCT)
US/PCT Application Serial No.	Filing Date	Status (patented, pending, abandoned)/ U.S. application no. assigned (For PCT)

[ ] In this continuation-in-part application, insofar as the subject matter of any of the claims of this application is not disclosed in the above listed prior United States or PCT international application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or Imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys and/or agents with full power of substitution and revocation, to prosecute this application, to receive the patent, and to transact all business in the Patent and Trademark Office connected therewith: John A. Diaz (Reg. No. 19,550), John C. Vassil (Reg. No. 19,098), Alfred P. Ewert (Reg. No. 19,887), David H. Pfeffer, P.C. (Reg. No. 19,825), Harry C. Marcus (Reg. No. 22,390), Robert E. Paulson (Reg. No. 21,046),

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[ ] I hereby authorize the U.S. attorneys and/or agents named hereinabove to accept and follow instructions from _____ _____ as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and/or agents and me. In the event of a change in the person(s) from whom instructions may be taken I will so notify the U.S. attorneys and/or agents hereinabove.

Full name of first inventor Attila T. LORINCZ

Inventor's signature* _____ date

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Inventor's signature* _____ date

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[ ] ATTACHED IS ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY THIRD AND SUBSEQUENT INVENTORS FORM.

* Before signing this declaration, each person signing must:

1. Review the declaration and verify the correctness of all information therein; and

2. Review the specification and the claims, including any amendments made to the claims.

After the declaration is signed, the specification and claims are not to be altered.

To the inventor(s):

The following are cited in or pertinent to the declaration attached to the accompanying application:

Title 37, Code of Federal Regulation, § 1.56

Duty to disclose information material to patentability.

(a) A patent by its very nature is affect with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in patent was cited by the Office or submitted to the Office in the manner prescribed by §§1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

Title 35, U.S. Code § 101

Inventions patentable

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Title 35 U.S. Code § 102

Conditions for patentability; novelty and loss of right to patent

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent,

(b) the invention was patented or described in a printed publication in this or foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States, or

(c) he has abandoned the invention, or

(d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate field more than twelve months before the filing of the application in the United States, or

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or

(f) he did not himself invent the subject matter sought to be patented, or

(g) before the applicant's invention thereof the invention was made in this country by another had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other ...

Title 35, U.S. Code § 103

Conditions for patentability; non-obvious subject matter

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Title 35, U.S. Code § 112 (in part)

Specification

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms also enable any person skilled in the art to which it pertains, or with which it is mostly nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Title 35, U.S. Code § 119

Benefit of earlier filing date in foreign country; right of priority

An application for patent for an invention filed in this country by any person who has, or whose legal representatives or assigns have, previously regularly filed an application for a patent for the same invention in a foreign country which affords similar privileges in the case of applications filed in the United States or to citizens of the United States, shall have the same effect as the same application would have if filed in this country on the date on which the application for patent for the same invention was first filed in such foreign country, if the application in

this country is filed within twelve months from the earliest date on which such foreign application was filed; but no patent shall be granted on any application for patent for an invention which had been patented or described in a printed publication in any country more than one year before the date of he actual filing of the application in this country, or which had been in public use or on sale in this country more than one year prior to such filing.

Title 35, U.S. Code § 120

Benefit or earlier filing date in the United States

An application for patent for an invention disclosed in the manner provided by the first paragraph of section 112 of this title in an application previously filed in the United States, or as provided by section 363 of this title, which is filed by an inventor or inventors named in the previously filed application shall have the same effect, as to such invention, as though filed on the date of the prior application, if filed before the patenting or abandonment of or termination of proceedings on the first application or an application similarly entitled to the benefit of the filing date of the first application and if it contains or is amended to contain a specific reference to the earlier filed application.

Please read carefully before signing the Declaration attached to the accompanying Application.

If you have any questions, please contact Morgan & Finnegan, L.L.P.

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